REMARKS

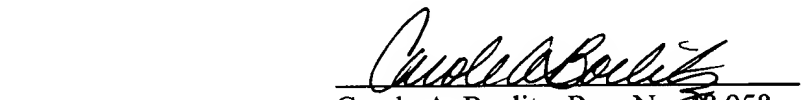
This preliminary amendment amends the specification, originally numbered 9-21 claims from pages 96-97, and adds new claims 25-37. Applicants correct minor typographical errors and the claim numbering, since claims 9, 10, and 11 were each used twice. No new matter has been added. Applicants respectfully submit that all of the pending claims, without amendment, are patentable. Accordingly, Applicants respectfully request allowance of all of the claims.

CONCLUSION

In view of the foregoing amendments and remarks, this application should now be in condition for allowance. A Notice of Allowance is respectfully requested. Applicant requests the Examiner to telephone Applicants' attorney if the Examiner believes the application is not in condition for allowance.

If this response is not considered timely filed and if a request for an Extension of Time is otherwise absent, Applicants hereby request any necessary Extension of Time. If there is a fee occasioned by this response, including an Extension Fee that is not covered by an enclosed check, please charge any deficiency to Deposit Account Number 23/2825.

Respectfully submitted,



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MARKED-UP SPECIFICATION

Please replace the paragraph beginning at line 13 on page 1 as shown.

Intra-articular tissues, such as the anterior cruciate ligament (ACL), do not heal after rupture. In [adddition] addition, the meniscus and the articular cartilage in human joints also often fail to heal after an injury. Tissues found outside of joints heal by forming a fibrin clot, which connects the ruptured tissue ends and is subsequently remodeled to form scar, which heals the tissue. Inside a synovial joint, a fibrin clot either fails to form or is quickly lysed after injury to the knee, thus preventing joint arthrosis and stiffness after minor injury. Joints contain synovial fluid which, as part of normal joint activity, naturally prevent clot formation in joints. This fibrinolytic process results in premature degradation of the fibrin clot scaffold and disruption of the healing process for tissues within the joint or within intra-articular tissues.

Please replace the paragraph beginning at line 15 on page 2 as shown.

Further provided by the invention is a tissue adhesive composition of collagen, an extracellular matrix protein, and a platelet formulated for the administration to a patient. Additionally, the invention provides a composition of collagen, a platelet and a neutralizing agent, *e.g.* sodium hydroxide or hydrochloric acid formulated for the administration to a patient. The patient is [is] preferably a mammal. The mammal can be, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow. In various aspects the platelet is derived from the patient. In other aspects the platelet is derived from a donor that is allogeneic to the patient.

Please replace the paragraph beginning at line 4 on page 3 as shown.

Alternatively, the composition includes one or more additives, such as insoluble collagen, a growth factor, a cross-linking agent, a stem cell, a genetically altered fibroblast and a cell media supplement. Growth factor [include] includes for example, platelet derived growth factor-AA (PDGP-AA), platelet derived growth factor-BB (PDGF-BB), platelet derived growth factor-AB (PDGF-AB), transforming growth factor beta (TGF-β), epidermal growth factor (EGF), acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), interleukin-1-alpha (IL-1α), and insulin.

Please replace the paragraph beginning at line 12 on page 3 as shown.

By cross-linking agent is meant that the agent is capable of forming chemical [binds] bonds between the constituents of the composition. The cross-linking agent can be for example, a protein or a small molecule, *e.g.*, glutaraldehyde or alcohol.

Please replace the paragraph beginning at line 21 on page 3 as shown.

The invention further provides a method of treating an extra-articular injury in a subject, by contacting the ends of a ruptured tissue from the subject with a composition of the invention. Extra-articular injuries include for example, injuries of the [is] ligament, tendon, bone or muscle.[.]

Please replace the paragraph beginning at line 17 on page 4 as shown.

The invention also includes the use of a collagen-based glue as an adhesive to maintain contact between the torn edges of the meniscus. The torn edges of the meniscus are pretreated to expose selected extracellular matrix components in the meniscus. Then, the glue is introduced into the tear. Bonds are formed between the extracellular matrix [of] in the meniscal tissue and the material of the glue. The bonds form a bridge across the gap in the meniscus. This adhesive zone bridge can then induce the migration of cells to the bridge, which is then remodeled by the meniscal cells, thus healing the tear.

Please replace the paragraph beginning at line 24 on page 4 as shown.

This invention further includes the use of a collagen-based scaffold as an adhesive, *e.g.* tissue-adhesive composition (as well as a cell migration inducer) to maintain and restore contact between the torn cartilage and the surrounding cartilage and bone. The torn edges are pretreated to expose the extracellular matrix components in the cartilage. A collagen scaffold (*e.g.* tissue-adhesive [composition)is]composition) is then introduced into the tear. Bonds are formed between the extracellular matrix of the torn tissue and the material of the glue. The bonds form a bridge across the gap in the articular cartilage. This adhesive zone bridge can then induce the migration of cells to the bridge, which is remodeled by the cartilage cells, thus healing the injured area.

Please replace the paragraph beginning at line 21 on page 5 as shown.

FIG. 4 is a schematic of tissue allocation for explants for 2-dimensional (2-D) and 3-dimensional ([2] 3-D) migration constructs.

Please replace the paragraph beginning at line 4 on page 6 as shown.

FIG. 9 is a histogram showing the cell densities in collagen-glycosaminoglycan ([GA] CG) matrices into which cells from explants from femoral, middle, and tibial zones of ruptured anterior cruciate ligaments migrated and proliferated after 1, 2, 3, and 4 weeks in culture. (Values are the [M]mean±SEM.)

Please replace the paragraph beginning at line 12 on page 6 as shown.

FIG. 12 is a schematic of the gross and histologic appearance of the four phases of the healing response in the human anterior cruciate ligament. FIG. 12A shows the inflammatory phase showing mop-ends of the remnants (a), disruption of the epiligament and synovial covering of the ligament (b), intimal hyperplasia of the vessels (c) and loss of the regular crimp structure near the site of injury (d). FIG. 12B shows the epiligamentous regeneration phase involving a gradual recovering of the ligament remnant by vascularized, epiligamentous tissue and synovium (e). FIG. 12C shows the proliferative phase with a revascularization of the remnant with groups of capillaries (f). FIG. 12D shows the remodeling and maturation stage characterized by a decrease in cell number density and blood vessel density (g), and retraction of the ligament remnant (h).

Please replace the paragraph beginning at line 12 on page 7 as shown.

[Fig.] FIG. 20 is a drawing illustrating preparation of the molds.

Please replace the paragraph beginning at line 13 on page 7 as shown.

[Fig.] FIG. 21 is a photomicrograph of the collagen gel with human ACL cells demonstrating increasing cell number density and increasing cellular alignment with time in culture. All micrographs are at 200X.

Please replace the paragraph beginning at line 17 on page 7 as shown.

[Fig.] FIG. 22[:] is a drawing illustrating the position of the explanted ACL tissue in the mold.

Please replace the paragraph beginning at line 19 on page 7 as shown.

[Fig.] FIG. 23[:] is a graph illustrating gel contraction with time in the gel with cells and the gel without cells.

Please replace the paragraph beginning at line 21 on page 7 as shown.

[Fig.] FIG. 24[:] is a photomicrograph of the interface between the ACL tissue (explant) and the gel in both the cell-gel and the cell-free gel after 21 days in culture.

Please replace the paragraph beginning at line 23 on page 7 as shown.

[Fig.] FIG. 25[:] is a photograph of a mold with mesh at one end and a needle to secure tissue at the other end.

Please replace the paragraph beginning at line 25 on page 7 as shown.

[Fig.] FIG. 26[:] is a graph illustrating minimum gel widths for the four groups during the four weeks of culture.

Please replace the paragraph beginning at line 27 on page 7 as shown.

[Fig.] FIG. 27[:] is a photomicrograph of the PRP gel at 1 mm from the explanted ACL tissue.

Please replace the paragraph beginning at line 29 on page 7 as shown.

[Fig.] FIG. 28[:] is a histogram demonstrating cell proliferation in a collagen scaffold with the addition of selected growth factors.

Please replace the paragraph beginning at line 31 on page 7 as shown.

[Fig.] FIG. 29[:] is a photomicrograph[s] of the collagen gel with human ACL cells.[d]

Please replace the paragraph beginning at line 1 on page 8 as shown.

[Fig.] FIG. 30[:] is a photomicrograph of the FGF-2 gel at 1 mm from the explanted ACL [tissue] tissue.

Please replace the paragraph beginning at line 3 on page 8 as shown.

[Fig.] FIG. 31[:] is a histogram demonstrating the effect of "growth factor cocktail" (GFC) concentration on retention of DNA in the ACL cell seeded gels after three weeks in culture.

Please replace the paragraph beginning at line 13 on page 8 as shown.

The compositions [ ]of the invention, can be incorporated into pharmaceutical compositions and administered to a subject[ ].

Please replace the paragraph beginning at line 15 on page 8 as shown.

The invention also provides methods of treating intra and extra articular injuries in a subject, *e.g.*, mammal by contacting the ends of a ruptured tissue from the subject with the compositions of the invention. Intra-articular injuries include for example, meniscal tears, ligament tears and cartilage lesion. Extra-articular injuries include for example[s] injuries to the ligament, tendon or muscle.

Please replace the paragraph beginning at line 18 on page 10 as shown.

Porous collagen scaffolds of varying composition and architecture have been researched as templates for regeneration of a variety of tissues including bone, skin and muscle. A porous collagen-glycosaminoglycan (CG) scaffold has been used successfully in regeneration of dermis (Yannas *et al.*, 86 Proc. Natl. Acad. Sci. USA 933-937 (1989)) and peripheral nerve (Chamberlain, *Long Term Functional And Morphological Evaluation Of Peripheral Nerves Regenerated Through Degradable Collagen Implants* (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library)).

Please replace the paragraph beginning at line 25 on page 12 as shown.

Cross-linking can be performed using chemicals, such as glutaraldehyde or alcohol, or physical methods, such as ultraviolet light or dehydrothermal treatment. The degree to which the properties of the scaffold are affected is dependent upon the method and degree of cross-linking. Cross-linking with glutaraldehyde has been widely used to alter the strength and degradation rate of collagen-based biomaterials scaffolds (Kato & Silver, 11 Biomaterials 169-175 (1990), Torres, *Effects Of Modulus Of Elasticity Of Collagen Sponges On Their Cell-Mediated Contraction In Vitro* (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library); Troxel, *Delay Of Skin*

Wound Contraction By Porous Collagen-GAG Matrices (Ph.D. Thesis Massachusetts Institute of Technology, 1994)(on file with the MIT Library)), and glutaraldehyde-cross-linked collagen products are commercially available for implant use in urologic and plastic surgery applications.

Please replace the paragraph beginning at line 10 on page 13 as shown.

The nonlinear relationship between stress and strain for scaffolds cross-linked using glutaraldehyde, dehydrothermal treatment, ultraviolet light irradiation and ethanol treatment has demonstrated higher stiffness in the ethanol and ultraviolet groups, lowest stiffness in the dehydrothermal cross-linked groups, with the stiffness of the glutaraldehyde group in between (Torres, *Effects Of Modulus Of Elasticity Of Collagen Sponges On* [*The*] *Their Cell-Mediated Contraction In Vitro* (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library)). Torres seeded collagen-based scaffolds with calf tenocytes and demonstrated a statistically significant increased rate of calf tenocyte cell proliferation in the glutaraldehyde and ethanol cross-linked scaffolds when compared with the dehydrothermal cross-linked group at 14 and 21 days post-seeding. Additional length of cross-linking in glutaraldehyde lead to increasing stiffness of the collagen scaffold, with values approaching that seen in the ultraviolet and ethanol groups. The ultraviolet cross-linked group demonstrated a statistically significant increase over the dehydrothermal group at 21 days, but not at 14 days post-seeding. This result suggests an influence of cross-linking method with fibroblast proliferation within the collagen-based scaffold.

Please replace the paragraph beginning at line 19 on page 13 as shown.

Cross-linking of collagen-based scaffolds affects the strength, biocompatibility, resorption rate, and antigenicity of these biomaterials (Torres, *Effects Of Modulus Of Elasticity Of Collagen Sponges On Their Cell-Mediated Contraction In Vitro* (M.S. Thesis Massachusetts Institute of Technology, 1998) (on file with the MIT Library); Troxel, *Delay of skin wound contraction by porous collagen-GAG matrices* (Ph.D Thesis Massachusetts Institute of Technology, 1994)(on file with the MIT Library); Weadock *et al.*, 29 J. Biomed. Mater. Res. 1373-1379 (1995)).

Please replace the paragraph beginning at line 3 on page 15 as shown.

For methods involving a collagen-based scaffold, the affected extremity is prepared and draped in the standard sterile fashion. A tourniquet may be used if indicated. Standard

arthroscopy equipment may be used. After diagnostic arthroscopy is performed, and the intra-articular lesion identified and defined, the tissue ends are pretreated, either mechanically or chemically, and the scaffold introduced into the tissue defect. The scaffold is then bonded to the surrounding tissue by creating chemical or mechanical bonds between the tissue proteins and the scaffold adhesive zone. This can be done by the addition of a chemical agent or a physical agent such ultraviolet light, a laser, or [heat,] heat. The scaffold may be reinforced by placement of sutures or clips. The arthroscopic portals can be closed and a sterile dressing placed. The post-operative rehabilitation is dependent on the joint affected, the type and size of lesion treated, and the tissue involved.

Please replace the paragraph beginning at line 22 on page 16 as shown.

The anterior cruciate ligament is a complex tissue composed of structural proteins, proteoglycans, and cells. The histology of the human anterior cruciate ligament is characterized by the specific distribution and density of the fibroblast phenotype as well as by the unique organization of the structural proteins. Three histologically different zones were found to be present along the anteromedial bundle from the femoral to the tibial attachment. Two of the zones (the fusiform and ovoid) were located in the proximal 1/3 of the bundle. The third zone (the spheroid) occupied the distal 1/3 of the bundle fascicles.

Please replace the paragraph beginning at line 14 on page 17 as shown.

In summary, cells expressing the α-sm actin isoform are present in the intact human anterior cruciate ligament, in cells with various morphologies, and predominantly in cells located at areas of crimp disruption. [The presence of α-sm actin positive, potentially contractile, cells in the human anterior cruciate ligament thus provides a possible explanation for the retraction of ligament remnants seen after rupture. Down-regulation of the myofibroblast phenotype may be useful preventing premature ligament retraction, while up-regulation may be useful in self-tensioning of the healed ligament during the remodelling phase.]

Please replace the paragraph beginning at line 22 on page 17 as shown.

The presence of α-sm actin positive, potentially contractile, cells in the ruptured human anterior cruciate ligament may provide one possible explanation for the retraction of ligament remnants seen after complete rupture. Down-regulation of the myofibroblast phenotype may be

useful in preventing premature ligament retraction, while up-regulation may be useful in self-tensioning of the healed ligament during the [remodelling] remodeling phase. Quantifying the degree of expression of the contractile actin and the effect of scaffold cross-linking and growth factors on this expression is a first step towards understanding possible regulation mechanisms.

Please replace the paragraph beginning at line 7 on page 18 as shown.

Methods. Fifteen intact anterior cruciate ligaments were obtained from total knee arthroplasty patients, ages 54 to 82 years. Four of the ligaments were used solely for histology and immunohistochemistry. The remaining ligaments were sectioned into fascicles that were divided transversely in the midsubstance to make explants. The highly porous collagen-glycosaminoglycan matrix, composed of type 1 bovine hide collagen and chondroitin-6-sulfate, was prepared by freeze-drying the collagen-glycosaminoglycan dispension as described by Murray & Spector, in *45th Annual Meeting, Orthopaedic Research Society, Anaheim, CA* (1999). The average pore size of the collagen-glycosaminoglycan scaffold was 100 μm. Sample of the collagen-glycosaminoglycan matrix was sandwiched between 2 explants and the construct was stabilized by suturing the explants to silicone tubing [(4 mm i.d.)] (4 mm i.d.). The constructs were cultured in media containing Dulbecco's DMEM/F12 with 10% fetal bovine serum, 2% penicillin streptomycin, 1% amphotericin B, 1% L-glutamine and 2% ascorbic acid. Samples were fixed in formalin after one to six weeks, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Immunohistochemistry using monoclonal antibodies to detect α-sm actin was also performed. Cell counts were taken at the edge of the scaffold for a cell density measure and the furthest distance traveled from the tissue/scaffold interface recorded for each sample.

Please replace the paragraph beginning at line 24 on page 21 as shown.

Collagen-Glycosaminoglycan Scaffold. The porous collagen-glycosaminoglycan scaffold used in this EXAMPLE has been used successfully in regeneration of dermis (Yannas, in *Collagen Vol III: Biotechnology*, Nimni, ed., p. 87-115 (CRC Press, Boca Raton, FL., 1989)) and peripheral nerve (Chamberlain, *Long Term Functional And Morphological Evaluation Of Peripheral Nerves Regenerated Through Degradable Collagen Implants* (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library)). The 3-D culture substrate was a highly porous collagen-glycosaminoglycan matrix, composed of type I bovine

tendon collagen (Integra Life Sciences, Inc., Plainsboro, NJ) and chondroitin-6-sulfate (Sigma Chemical, St.. Louis, MO). The scaffold was prepared by freeze-drying the collagen-glycosaminoglycan dispersion under specific freezing conditions described by Yannas *et al,* 8 Trans. Soc. Biomater. 146 (1985) to form a tube with pore channels preferentially oriented longitudinally. The average pore size of the collagen-glycosaminoglycan scaffold manufactured in this manner has previously been reported by Louie, *Effect Of A Porous Collagen-Glycosaminoglycan Copolymer On Early Tendon Healing In A Novel Animal Model* (Ph.D. Thesis Massachusetts Institute of Technology, 1997)(on file with the MIT Library) as 100 μm.

Please replace the paragraph beginning at line 26 on page 23 as shown.

Histology of the Ligament Fascicles. The histology of the fascicles from each of the 6 patients was as follows: The proximal 1/3 was populated predominantly by fusiform and ovoid cells in relatively high density, and the distal [2/3was] 2/3 was populated by a lower density of spheroid cells. The level of transection used to produce the fascicle constructs was in the spheroid cell region, with similar cell morphologies and an average cell number density of 498±34 cells/mm^2 (n=6). α-sm actin immunohistochemistry of the transected region showed positive staining in 8.3±3.0% of fibroblasts not associated with blood vessels.

Please replace the paragraph beginning at line 4 on page 24 as shown.

Changes in the Fascicular Tissue with Time in Culture. With time in culture, changes in the cell distribution and extracellular matrix organization of the anterior cruciate ligament tissue in the 36 test and control fascicular constructs were observed. Fusiform, ovoid and spheroid nuclear cell morphologies could be observed in the bulk of the cultured fascicles. Time in culture was noted to have a statistically significant effect on the cell number density at each location (*i.e.*, at the edge and at 1 and 2 mm into the bulk of the fascicle; one-way ANOVA, $p < 0.001$). The number density of cells at the edge of the explants decreased to 120±29 cells/mm^2 at 2 weeks and to 101±28 cells/mm^2 at six weeks, both of which were different from the cell number density at retrieval, [498∀34] 498±34 cells/mm^2, as noted above (paired t-test, $p < 0.001$). The number of cells within the bulk of the fascicle decreased as well, to 58±21 cells/mm^2 at 2 weeks and 19±20 cells/mm^2 at six weeks, again, both densities were significantly different from that at retrieval (paired t-test, $p < 0.0001$).

Please replace the paragraph beginning at line 14 on page 25 as shown.

In the constructs with interposed collagen-glycosaminoglycan scaffolding, fibroblasts were noted to migrate from the human anterior cruciate ligament fascicles into the scaffolds at the earliest time point (2 weeks). Migration into the scaffold was seen in 5 of 6 constructs at 2 weeks, 5 of 6 constructs at 4 weeks, and in all 5 of the 6-week constructs. While the average cell number density in the fascicle decreased with time, the average cell number density in the scaffold increased with time in culture (FIG. 7). Initially, cells were noted predominantly at the edge of the scaffold. With time, the average cell number density at the edge of the scaffold increased from 57±22 cells/mm^2 at 2 weeks and to [120∀41] 120±41 cells/mm^2 at six weeks. While this was a 2-fold increase, it was not found to be statistically significant ($p=0.15$) owing to the large coefficient of variation. The average cell number density 1 mm within the scaffold also increased from 6±2 cells/mm^2 at 2 weeks to 25±10 cells/mm^2 at 4 weeks and to 47±37 cells/mm^2 at 6 weeks. Again, owing to the large variation, these increases were not statistically significant ($p=0.15$), despite being increases of several-fold. While there was a consistent increase in the mean value of the cell number density with time at the various distances from the scaffold/fascicle interface, two way ANOVA showed no significant effect of time in culture on cell number density at each location ($p=0.10$), but did reveal a significant effect of location on cell number density ($p<0.001$). The maximum cell number density of fibroblasts in the scaffold increased with time from 123±45 cells/mm^2 at 2 weeks to 336±75 cells/mm^2 at six weeks, a difference which was statistically significant (Student t test, $p = 0.05$). The relationship between maximum cell number density and time was well modeled by a linear regression, with a coefficient of determination of 0.96 (FIG. 8). Cells migrating into the collagen-glycosaminoglycan scaffold demonstrated all of the three previously described ligament fibroblast morphologies: (1) fusiform or spindle-shaped, (2) ovoid, and (3) spheroid. The average migration distance at the 2-week time period was 475 micrometers. At the 4-week time point, cells had migrated as far as 1.5 mm toward the center of the scaffold. In areas where a gap greater than 50 microns was present between the explant and collagen-glycosaminoglycan scaffold, no cell migration into the scaffold was seen.

Please replace the paragraph beginning at line 23 on page 30 as shown.

Cross-linking. All of the 3-D collagen-glycosaminoglycan scaffolds are minimally cross-linked using dehydrothermal treatment at 105°C and 30 mtorr for 24 hr. Additional cross-linking is performed for the glutaraldehyde, ultraviolet, and ethanol groups. Glutaraldehyde cross-linking are performed by rehydrating the collagen-based scaffolds in acetic acid, treating in 0.25% glutaraldehyde for thirty minutes, rinsing and storing in a buffer solution. Ethanol cross-linking is performed by soaking the collagen scaffolds in 70% ethanol for 10 min, rinsing, and storing in buffer. Ultraviolet light cross-linking is performed by placing the scaffold 30 cm from an ultraviolet lamp rated at 5.3 W total output, 55.5 W/cm^2 at 1 m. The scaffolds is cross-linked for 12 hr, 6 hr on each side as previously described by Torres, *Effects Of Modulus Of Elasticity Of Collagen Sponges On* [*The*] Their *Cell-Mediated Contraction In Vitro* (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library).

Please replace the paragraph beginning at line 16 on page 31 as shown.

Histology for analysis of cell migration. All specimens for light microscopy, including control fascicles and explants are fixed in 10% neutral buffered formalin for one week, embedded in paraffin and sectioned into 7 micrometer sections. Sections are taken perpendicular to the explant/scaffold interface to allow for migration measurements. Hematoxylin and eosin staining are performed to facilitate light microscopy examination of cell morphology in both explant and [scaffold.]scaffold, maximum migration distance into the collagen-glycosaminoglycan scaffold and maximal number density of fibroblasts in the scaffold.

Please replace the paragraph beginning at line 24 on page 31 as shown.

DNA Assay for Cell Proliferation. Specimens allocated for analysis of DNA content are fluorometrically. Specimens are rinsed in phosphate-buffered saline and the explant separated from the scaffold. The scaffold is stored at -70°C. The [scaffolds] scaffold is digested in 1 ml of 0.5% papain/buffer solution in a 65°C water bath. A 200 µl aliquot of the digest is combined with 40 µl of Hoechst dye no. 33258 and evaluated fluorometrically. The results are extrapolated from a standard curve using calf thymus DNA. For one run of the DNA assay, a standard curve based on a sample of human ligament cells are used to estimate the cell number from the DNA measurement. Negative control specimens consisting of the scaffold material alone are also assayed to assess background from the scaffold.

Please replace the paragraph beginning at line 16 on page 35 as shown.

Results. Cells were noted to migrate from the anterior cruciate ligament rupture site into the scaffold at the earliest time point (two weeks). Higher densities of cells were noted to migrate from explants obtained at the site of rupture than from explants taken far from the rupture site, or from the intact anterior cruciate ligaments (FIG. 10). Two-way ANOVA demonstrated explant location in the ligament had a significant effect on cell number density in the scaffold for the ruptured ligaments ($p<0.0001$), but that time in culture did not have a significant effect. Maximum cell number densities in the scaffold (335±200 [cells/mm^2)] cells/mm^2).

Please replace the paragraph beginning at line 9 on page 39 as shown.

Material and methods. Samples of articular cartilage were obtained from 15 patients undergoing total joint arthroplasty for osteoarthrosis. While the specimens were obtained from patients with joint pathology, areas of cartilage with no grossly noticeable thinning, fissuring, or fibrillation were selected. Using a dermal punch, [cylindrincal] cylindrical samples (4.5mm diameter and 2-3 mm thick), were cut from the specimens. Explants were cultured in 6-well culture dishes and oriented so that deep zone of the tissue contracted the culture dish. In the first test, 20 cartilage samples were obtained from each of the 9 patients. Four plugs of cartilage were allocated to one of five groups that received collagenase treatment for 0, 1, 5, 10, or 15 min. The time to cell attachment after outgrowth was determined and cultures were terminated after 28 days. From 6 of the 9 patients, additional plugs, untreated and treated with [collagease] collagenase for 15 minutes, were evaluated for α-sm, immediately after treatment, and at 6, 14 and 20 days in culture. In the second test, 24 cartilage plugs were obtained from each of 6 additional patients. Four plugs were allocated to 5 groups receiving a different enzymatic treatment for 15 min. and a sixth untreated control group: (a) 380 U/ml clostridial collagenase (0.1%; Sigma Chemical, St.[.] Louis, MO); (b) 1100 U/ml hyaluronidase (0.1%; Sigma Chemical); (c) 1 U/ml chondroitinase ABC (Sigma Chemical), (d) 0.05% trypsin (Life Technologies); and (e) 1100 U/ml hyaluronidase followed by 380 U/ml collagenase (7.5 min. in each). The days when cell outgrowth (round cells separated from the explant) and cell attachment (elongated cells) were first evident were recorded. All cultures were terminated after 30 days. If no outgrowth was noted, time to outgrowth was assigned 28 or 30 days for exps. 1 and 2, respectively. Explants allocated for immunohistochemistry were fixed in 10% formalin, paraffin embedded and cut to 7 μm sections. Sections were stained with a α-sm monoclonal

antibody (Sigma Chemical, St.[.] Louis, MO). Statistical analysis was performed by ANOVA with Fisher's PLSD post-hoc test.

Please replace the paragraph beginning at line 7 on page 40 as shown.

Treatments with hyaluronidase, chondroitinase ABC, and trypsin[ ], had no effect on the times to outgrowth and attachment (TABLE 3). In [contract] contrast, the collagenase treatment yielded a time to outgrowth of at least 1 order of magnitude less than the untreated group (2.2±0.2 vs 27.7±1.5 days, respectively; TABLE 3). Treatment of the explants with hyaluronidase + collagenase yielded results that were comparable to treatment with collagenase alone. Signs of attachment of the outgrowth cells were generally found within 3 days of the first evidence of outgrowth.

Please replace the paragraph beginning at line 15 on page 46 as shown.

Most of the changes [occured] occurred in the epiligament that displayed an increase in cell number density and blood vessel density. The vascular epiligamentous tissue was noted to gradually extend over the ruptured ligament end, encapsulating the mop-ends of the individual capsules. Thickening of the epiligament and fibroblast proliferation were seen to occur during this time period. A synovial layer, similar to that seen covering the epiligamentous tissue in the intact anterior cruciate ligament, was noted to form over the extending neoepiligamentous tissue.

Please replace the paragraph beginning at line 1 on page 54 as shown.

The 3-D culture substrate used in this EXAMPLE was a highly porous collagen-glycosaminoglycan matrix, composed of type I bovine hide collagen and chondroitin-6-sulfate, prepared by freeze-drying the collagen-glycosaminoglycan dispersion under specific freezing conditions (Yannas *et al.*, 8 Trans Soc Biomater. 146 (1985)) to form a tube with pore orientation preferentially oriented, longitudinally. The average pore size of the collagen-glycosaminoglycan scaffold manufactured in this manner has previously been reported as 100 gm (Chamberlain, *Long Term Functional And Morphological Evaluation Of Peripheral Nerves Regenerated Through Degradable Collagen Implants*. (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library)).

Please replace the paragraph beginning at line 12 on page 55 as shown.

3-D Culture Outgrowth. In the constructs with interposed collagen-glycosaminoglycan scaffolding, fibroblasts migrated from the human anterior cruciate ligament explants into the templates at the earliest time point (1 week). At one week, migration into the templates was seen in 4 of 4 of the templates cultured with explants from the injury zone, [I]1 of 4 templates cultured with explants from the middle zone, and 1 of 4 of the templates cultured with explants from the normal zone. By four weeks, cells were seen in 3 of 3 templates cultured with the injury zone explants (the fourth template had been completely degraded) and in 3 of four of the templates cultured with the normal zone explants. Five of the explants completely degraded the template prior to the collection time. The location from which the explants were taken (injury, middle or normal) was found to have a statistically significant effect on the cell number density in the template (two way ANOVA, $p = 0.001$), with Bonferroni-Dunn post-hoc testing demonstrating differences between templates cultured with explants from the injury zone and middle zone ($p=0.009$) and the injury and normal zone ($p=0.003$; FIG. 16). The difference between the template cell density for templates cultured with explants from the middle and tibial of the twelve explants (three from the injury zone, two from the middle zone, and two from the normal zone) demonstrated confluent growth for at least two consecutive time periods prior to termination and were included in the calculation of the average rate. All explanted tissue and fibroblasts on the culture wells were fixed in formalin after four weeks in culture.

Please replace the paragraph beginning at line 17 on page 57 as shown.

The outgrowth rates noted for the explants from ruptured ligaments was found to be about 0.25 mm/day. However, the average time to outgrowth was four days shorter for the ruptured anterior cruciate ligament explants (6.6 ± 2.0 days) than that reported for the intact anterior cruciate ligament explants (10 ± 3 days) (Murray *et al.*, 17(1) J. Orthop. Res. 18-27 (1999)).

Please replace the paragraph beginning at line 14 on page 64 as shown.

Assay design. The assay design is similar to that of EXAMPLE 4. Human anterior cruciate ligament explants are obtained from patients undergoing total knee arthroplasty. Ligaments which are grossly disrupted or demonstrate gross signs of fatty degeneration are excluded from the analysis. A fairly uniform distribution of cells occurs in the distal 2/3 of the ligament fascicles, so this section is used for all assays. The preparation of the collagen-based

scaffold is as described in EXAMPLE 4 and previously reported by Torres, *Effects Of Modulus Of Elasticity Of Collagen Sponges On Their Cell-Mediated Contraction In Vitro* (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library). The cross-linking of the scaffolds is as described in EXAMPLE 4 and as previously described by Torres, *Effects Of Modulus Of Elasticity Of Collagen Sponges On Their Cell-Mediated Contraction In Vitro* (M.S. Thesis Massachusetts Institute of Technology, 1998)(on file with the MIT Library). The growth factors are added to the cell culture media as described in EXAMPLE 4. Culture, histology for analysis of cell migration, DNA assay for cell proliferation, immunohistochemistry for the contractile actin isoform, and SDS-PAGE analysis for the synthesis of type I collagen are as described in EXAMPLE 4. A pilot assay is performed to assess the DNA content with the DHT cross-linked scaffold with the addition of no growth factors. Alternatively, a tritiated thymidine assay can be evaluated or the specimens used for proliferation can be fixed and serially sectioned, with sections at regular intervals examined for cell number density. Maximum number density is recorded for each specimen type. Associated histology is used to estimate the percentage of dead cells.

Please replace the paragraph beginning at line 12 on page 66 as shown.

Military Significance. In a recent study of midshipmen attending the U.S. Naval Academy, the incidence rate of anterior cruciate ligament (ACL) injury was 10 times higher for women than men (Gwinn *et al.*, *Relative gender incidence of anterior cruciate ligament injury at a military service* [*academy.*] *academy,* in *66th Annual* [*Meeting.*] *Meeting,* Anaheim, CA (1999)). In military related training, the incidence of anterior cruciate ligament rupture was 6 times higher that in competitive, high risk sports. The study also found that women engaged in military training sustained an anterior cruciate ligament tear 3 times per every 1000 exposures. Thus, for women engaged in military training exercises twice a week, an average of 1 in 4 will sustain an anterior cruciate ligament tear each year (Gwinn *et al.*, *Relative gender incidence of anterior cruciate ligament injury at a military service academy*[.], in *66th Annual* [*Meeting.*] *Meeting,* Anaheim, CA (1999)). This study, and others, highlight the importance of anterior cruciate ligament rupture in women, particularly women engaged in activities which place them at risk for this injury, such as military training. More than 200,000 people rupture their anterior cruciate ligament annually (*National Center for Health Statistics* (1986)), and the risk of anterior cruciate ligament rupture is significantly higher for women engaged in intercollegiate sports

when compared with their male counterparts (Arendt & Dick, 23(6) Am. J. Sports Med. 649-701 (1995), Stevenson, 18 Iowa Orthop. J. 64-66 (1998)). For many women athletes, anterior cruciate ligament rupture may be a career-ending injury, as many patients can not return to their previous level of activity, even after repair or reconstruction (Marshall *et al.*, 143 Clin Orthop 97-106 (1979); Noyes *et al.*, 68B J. Bone Joint Surg. 1125-1136 (1980)). Development of new methods of treatment of the ruptured anterior cruciate ligament, including ligament regeneration, may lead to quicker recovery times and improved rates of return to high levels of physical training for both women and men.

Please replace the paragraph beginning at line 15 on page 68 as shown.

Collagen-glycosaminoglycan (CG) scaffold synthesis. The scaffold used in this EXAMPLE is the same scaffold used in EXAMPLE 3. The 3-D culture substrate is a highly porous CG matrix, composed of type I bovine hide collagen and chondroitin-6-sulfate. This is prepared by freeze-drying the collagen-glycosaminoglycan dispersion under specific freezing conditions (Louie, *Effect of a porous collagen-glyosaminoglycan copolymer on early tendon healing in a novel animal model* (Ph.D. Thesis Massachusetts Institute of Technology 1997)(on file with the MIT Library)). The average pore size of the CG scaffold manufactured in this manner is 100 μm.

Please replace the paragraph beginning at line 14 on page 69 as shown.

Knees undergoing primary repair with the placement of the scaffold in the defect between ruptured ligament ends have sutures placed in an identical manner to that in the primary repair group. The CG [scaffolds] scaffold is placed into the defect prior to tensioning of the sutures.

Please replace the paragraph beginning at line 11 on page 71 as shown.

(3) *Composite structure*: (a) laminate structure; (b) thickness of each ply; (c) number of plies; (d) orientation and stacking sequence of plies; (e) symmetry of the layup; (f) position of reinforcement within the matrix; (g) location within the part; (h) 3 dimensional orientation; (i) fiber density (e.g., distance between reinforcement components or reinforcement[:] matrix volume and weight ratios); (j) fiber contacts and cross-overs per mm; (k) reinforcement structure; (l) cross-sectional shape (m) surface texture and treatment; (n) dimensions; (o) fiber twist; (p) denier; (q) weave; (r) coating; (s) total number of coating layers; (t) thickness of each

layer; (u) voids; (v) mean volume percent; (w) interconnections; (x) penetration depth and profile; and (y) drawing or photographs of the product illustrating the position of the coating and any variation in coating thickness (*for example, see,* FIGS.) The anatomical location and attachment mechanism for the biological implant of the invention is provided in diagrams, illustrations, or photographs of the implant *in situ.*

Please replace the paragraph beginning at line 17 on page 74 as shown.

The ability of cells of the human anterior cruciate ligament to survive in a collagen hydrogel was assesed. Human anterior cruciate ligament was obtained from a patient undergoing total knee arthroplasty. The ligament was sectioned into 18 explants, each 1-2 mm on a side. The explants were then cultured in a 6 well plate with 1.5 cc of media/well containing high-glucose DMEM, 10% FBS and antibiotics. Media were changed three times a week. After four weeks of culture, the tissue was removed and the cells which had grown out of the tissue onto the plate were trypsinized, counted (1x 10^7 cells) and placed into [2] two 75 [cm^2] cc flasks overnight. On the second day, the gel components were assembled. All ingredients were kept on ice until placed into the molds. The molds were made by cutting 6 mm ID silicon tubing into 1["] inch lengths, then cutting each tube in half to make a trough. Silicon adhesive was then used to secure a piece of polyethylene mesh to each end of the trough (Figure 20). The adhesive was allowed to cure overnight, then sterilized by placing into sterile 70% EtOH for 2 hours. The molds were exhaustively rinsed in dIH20 and placed individually into 6 well plates prior to adding the gel. Prior to gel assembly, the cells were again trypsinized and centrifuged. The media was aspirated, leaving a pellet of cells in a 15 cc centrifuge tube. The gel was made by mixing 3.5 cc of acid-soluble, Type I collagen (Cell-A-Gen 0.5%, ICN Pharmaceuticals) with 1 cc of 10X Ham's F10, 1 cc of PCN/Strep, 0.1 ml Fungizone, 3 microliters of bFGF and 3.7 ml of sterile, distilled water. The above mixture was vortexed, and 1.4 ml of Matrigel added. The mixture was vortexed again, and then 0.155 cc of 7.5% NaOH was added. The mixture was vortexed, and added to the tube containing the cell pellet. The cells were resuspended in the cold gel by gentle mixing with a 1 cc pipette. The gel-cell mixture was then aliquoted into the molds, with 300 µl used in each mold. A drop of the gel-cell mixture was also placed into the bottom of each well to monitor cell survival in the gel. The constructs were allowed to sit at room temperature for 30 minutes, then moved to the 37 degree incubator for 30 minutes. After 1 hour, media containing 10% FBS was added to cover the mold and gel. Constructs were

sacrificed for histology at 3 hours, 3 days and 9 days. The gels were fixed in cold paraformaldehyde for 4 hours, then stored in PBS. The gels were embedded in paraffin and 7 micrometer sections cut. Serial sections were stained with hematoxylin and eosin and Masson's trichrome.

Please replace the paragraph beginning at line 1 on page 76 as shown.

The ability of endogenous or exogenous human anterior cruciate ligments cells to mediate collagen hydrogel contraction was assessed. Human ACL explants were cultured as in EXAMPLE 17 to obtain primary outgrowth human ACL cells. The cells were trypsinized from 9 wells (1.5 plates, approx 6 x 10^6 cells), and collected in a pellet as in Experiment 1. Additional explants were obtained from the ACL of a second patient undergoing arthroplasty on 12-4-00 (the day before the experiment was started.) Explants were 2 mm on each side. The explants were predigested in 0.1% collagenase for 15 minutes at 37 degrees C and then rinsed exhaustively in sterile PBS and placed in culture media which included 10% FBS. Explants were maintained in culture media at 37 degrees C and 5% CO2 overnight. The molds were made by sectioning the 6 mm ID silicon tubing in half to make a trough, and sealing the ends of the trough with agarose, which was sterilized by autoclaving. The agarose was melted by placing it in a [80degree] 80 degree C water bath, then 1 drop was added to each end of the mold. The molds were sterilized by placing in 70% EtOH for 2 hours, then rinsing exhaustively in sterile H2O. Each mold was placed into individual wells of a 6 well plate. One explant was placed into each end of the trough (Figure 22). A total of 18 constructs were prepared. Each mold was able to hold 200 microliters of liquid.

Please replace the paragraph beginning at line 11 on page 78 as shown.

For the gel without cells (groups 1 and 2), the gel was prepared as in EXAMPLE 17 and 18. A sterile pipette was used to add 300 microliters of gel to each mold. For the fibroblast gel (group 3), we trypsinized cells from [2] two 75 [cm2] cc flasks and resuspended these cells in 10 cc of gel prepared as in EXAMPLE 17 and 18

Please replace the paragraph beginning at line 19 on page 79 as shown.

The resiliency of the platelet rich plasma collagen hydrogels were assessed using a cyclic stretching machine. Explants were made as in EXAMPLES 17 and 18. The explants were

connected by a 3-0 nylon suture loop to prevent excessive tension in the gels. The explants were placed into molds, as in EXAMPLE[S] 18, and the gap between filled with either the gel used in experiments EXAMPLES 17 and 18 (standard gel) or the PRP gel of EXAMPLE 19. Eight constructs were used in each group. After the standard gel had been added to the constructs, it was allowed to set up for 60 minutes at room temperature and media added. For the PRP group, the gel was allowed to set up for 30 minutes at room temperature. After setting, the constructs were transferred into a cyclic stretching machine and cultured for 18 days.

Please replace the paragraph beginning at line 12 on page 80 as shown.

To determine the optimal concentration of PRP and matrigel to use in the collagen hydrogel [ge] gel without altering the cell proliferation rates or collagen production rate the following experiments were conducted. Primary outgrowth cells were obtained from one patient undergoing TKR as in EXAMPLE 17. Constructs were made as in EXAMPLE 17. One of five types of gel were added to the molds. The five gel groups were

6. Collagen Hydrogel (standard as used in Expts 1, 2, 3 and 4 – contains Matrigel)
7. Group 1 + 15% PRP
8. Group 1 + 30% PRP
9. Group 1 + 45% PRP
10. Group 3 without Matrigel

Twenty constructs for each group were cultured and four sacrificed at 2 hours, 1 day, 1 week, 2 weeks and 3 weeks of culture. One construct for each group at each time [pointwas] point was reserved for histology, and the other three labeled with tritiated thymidine (to measure cell proliferation) and 14C proline (to measure collagen production) for 24 hours prior to sacrifice. Minimum gel width was measured each week for all constructs.

Please replace the paragraph beginning at line 26 on page 87 as shown.

The effect of increased construct viscosity on gel retention in the ACL defect is determined using canine knees obtained at the time of sacrifice. All knees have partial transections in the ACL. Knees are treated with the control gel, or gels containing increasing amounts of insoluble collagen fiber. The degree of gel retention is assessed both grossly and histologically. To expose the ACL, a paramedian arthrotomy along the medial border of the

patellar tendon is made. The fat pad is [ ]swept laterally to expose the ACL. A partial defect [ismade] is made in the ACL using a transverse cut. After preparation of the defect, the gel components will be mixed as decribed in [EXAMPPLE] EXAMPLE 23.

Please replace the paragraph beginning at line 13 on page 91 as shown.

[Effects Of The Addition Of Growth Factors On The Fibroinductive Properties Of A Collagen Scaffold.] EFFECTS OF THE ADDITION OF GROWTH FACTORS ON THE FIBROINDUCTIVE PROPERTIES OF A COLLAGEN SCAFFOLD.

Please replace the paragraph beginning at line 4 on page 92 as shown.

EXAMPLE [26]27

SURVIVAL OF HUMAN [HUMAN] ANTERIOR CRUCIATE LIGAMENT CELLS IN FGF-2 SUPPLEMENTED COLLAGEN GEL

Please replace the paragraph beginning at line 19 on page 92 as shown.

EXAMPLE [27]28

MIGRATION OF HUMAN [HUMAN] ANTERIOR CRUCIATE LIGAMENT CELLS IN FGF-2 SUPPLEMENTED COLLAGEN GEL

Please replace the paragraph beginning at line 5 on page 93 as shown.

EXAMPLE [28]29

DETERMINATION OF THE OPTIMAL CONCENTRATION OF "GROWTH FACTOR COCKTAIL" (GFC) TO USE IN THE GEL FOR MAXIMUM STIMULATION OF CELL PROLIFERATION AND COLLAGEN PRODUCTION.

Please replace the paragraph beginning at line 9 on page 93 as shown.

Primary outgrowth cells were obtained from one patient undergoing TKR. Constructs were made as decribed in EXAMPLE [26]27 and [27]28. One of four types of gel were added to the molds. The four gel groups were

[6] 1. Collagen Hydrogel with FGF-2 only

[7] 2. Group 1 + 15% GFC

[8] 3. Group 1 + 30% GFC

[9] 4. Group 1 + 45% GFC

Please insert the following paragraph after line 4 on page 94 as shown.

What is claimed is:

MARKED-UP CLAIMS

[9] 12. (Amended) The tissue-adhesive composition of claim [8] 11, wherein said platelet is derived from said patient.

[10] 13. (Amended) The tissue-adhesive composition of claim [8] 11, wherein said composition further comprises plasma.

[11] 14. (Amended) The tissue-adhesive composition of claim [10] 13, wherein said plasma is derived from said patient.

[12] 15. (Amended) The tissue-adhesive composition of claim [8] 11, wherein said collagen is selected from the group comprising collagen type I, collagen type II, collagen type III, collagen type IV, collagen type V, collagen type IX and collagen type X.

[13] 16. (Amended) The tissue-adhesive composition of claim [8] 11, wherein said collagen is acid soluble type I collagen.

[14] 17. (Amended) A tissue-adhesive composition formulated for administration to a patient, comprising soluble type I collagen, an extracellular matrix protein, and a platelet derived from said patient.

[15] 18. (Amended) The tissue-adhesive composition of claim [3] 17, wherein said composition further comprises plasma derived from said patient.

[16] 19. (Amended) A method of treating an intra-articular injury in a subject, the method comprising: contacting the ends of a ruptured tissue from the subject with [the composition of claim 1 or 7] a composition comprising soluble type I collagen, a platelet, and at least one of an extracellular protein and a neutralizing agent.

[17] 20. (Amended) The method of claim [16] 19, wherein the intra-articular injury is a meniscal tear, ligament tear or a cartilege lesion.

[18] 21. (Amended) The method of claim [16] 19, further comprising mechanically joining the ends of the ruptured tissue.

[19] 22. (Amended) A method of treating an extra-articular injury in a subject, the method comprising contacting the ends of a ruptured tissue from the subject with [the composition of claim 1 or 7] a composition comprising soluble type I collagen, a platelet, and at least one of an extracellular protein and a neutralizing agent.

[20] 23. (Amended) The method of claim [16] 22, wherein the extra-articular injury is a ligament, tendon or muscle injury.

[21] 24. (Amended) The method of claim [16] 22, further comprising mechanically joined ends of the ruptured tissue.